Theses of doctoral (Ph.D.) dissertation

DEVELOPMENT OF ISOCYANIDE-BASED ONE-POT METHODS

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1. Introduction and Aims

One of the major aims of organic chemistry research and development is to improve the efficacy of synthetic methods either by the realization of convergent or divergent reaction routes or by the development and optimization of novel, more effective reagents or reactions. One-pot procedures are such highly efficient synthetic methods where multiple consecutive reaction steps are performed in a single reactor without the isolation of intermediates. In terms of implementation, sequential and tandem protocols can be distinguished. In a sequential one-pot procedure, the formation of each intermediate could be followed by the introduction of additional reagents or changing reaction conditions. In the case of a tandem reaction, however, one reaction step automatically induces the next in a consecutive fashion without the need to add any further reagent. In the group of tandem transformations, in multicomponent reactions (MCRs), at least three starting components are combined in order to form a complex product including all or the majority of atoms of starting materials in a single step via multiple consecutive substeps. Multicomponent reactions have a privileged place in the toolbox of medicinal chemistry, since they enable the rapid and on-demand automated construction of large and diverse molecular libraries by simply varying the starting compounds.

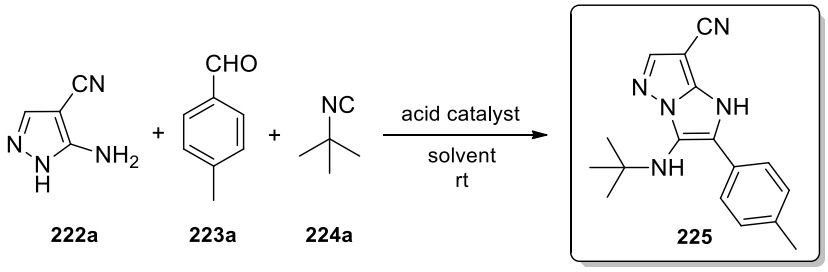
The aims of this doctoral dissertation were, on the one hand, isocyanide-based multicomponent synthesis of novel imidazo[1,2-*b*]pyrazoles with anticipated antitumor activity and, on the other hand, the development of an isocyanide-based novel one-pot sequential synthetic method towards *N,N'*-disubstituted guanidines.

2. Materials and Methods

In the course of the synthetic work, the majority of the reactions were performed in a millimolar scale. Reactions were monitored either by thin-layer chromatography or HPLC analyses. Products were purified by column chromatography (silica, alumina) or by simple filtration and recrystallization. The molecular structures of products were determined by one- and two-dimensional NMR techniques combined with mass spectrometric measurements.

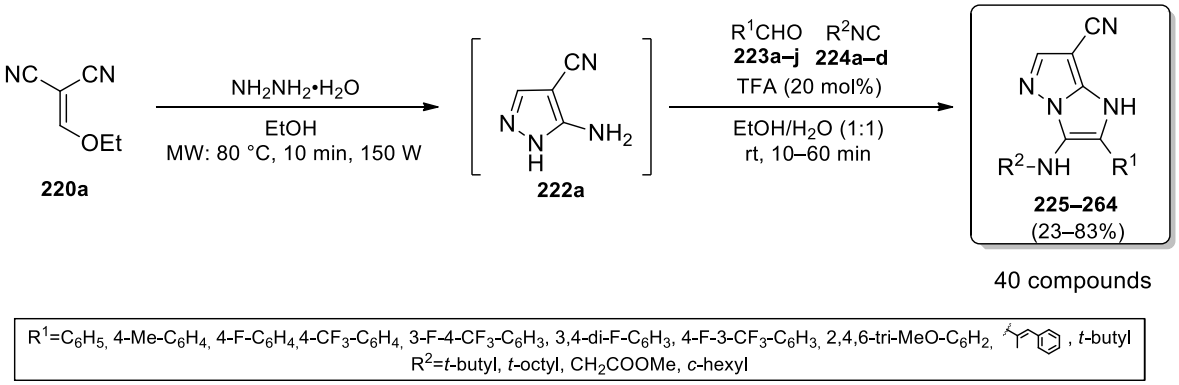
3. Results and Discussion*

3.1. Initially, the Groebke-Blackburn-Bienaymé three-component (GBB-3CR) reaction of 5-aminopyrazole-4-carbonitrile (**222a**) was investigated. Optimal reaction conditions (20 mol% TFA, EtOH/water 1:1, rt, 15 min) were set in a model reaction [*p*-tolualdehyde (**223a**) and *tert*-butyl isocyanide (**224a**) reactants; Scheme 1] testing Brønsted and Lewis acids in varied solvents and varying catalyst loadings.



Scheme 1

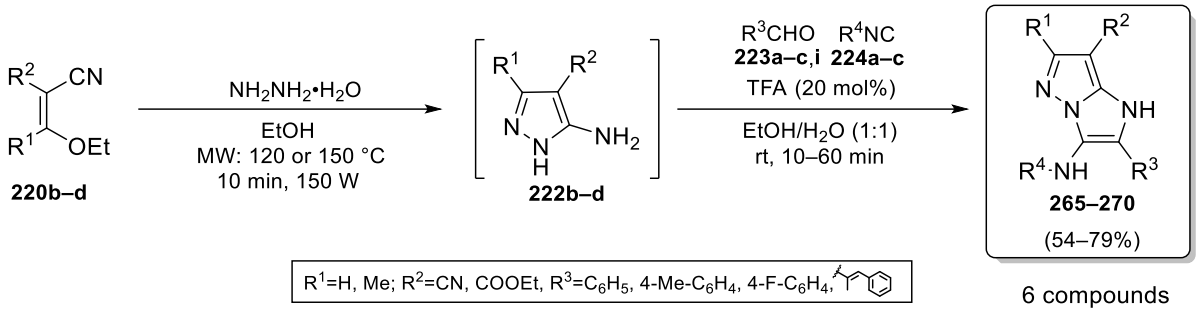
3.2. By combining the *in situ* preparation of aminopyrazole **222a** with the optimized GBB reaction step, 40 novel imidazo[1,2-*b*]pyrazole-7-carbonitrile derivatives (**225**–**264**) were synthesized in a sequential one-pot two-step procedure (Scheme 2). Utilizing aromatic and aliphatic aldehydes (**223a**–**j**) together with primary, secondary and tertiary aliphatic isocyanides, bicycles **225**–**264** were gained in low to good yields (23–83%). Considerable substituent effect was not observed, albeit, upon applying methyl isocyanoacetate, lower yields were achieved accompanied by side-product formation to a larger extent.



Scheme 2

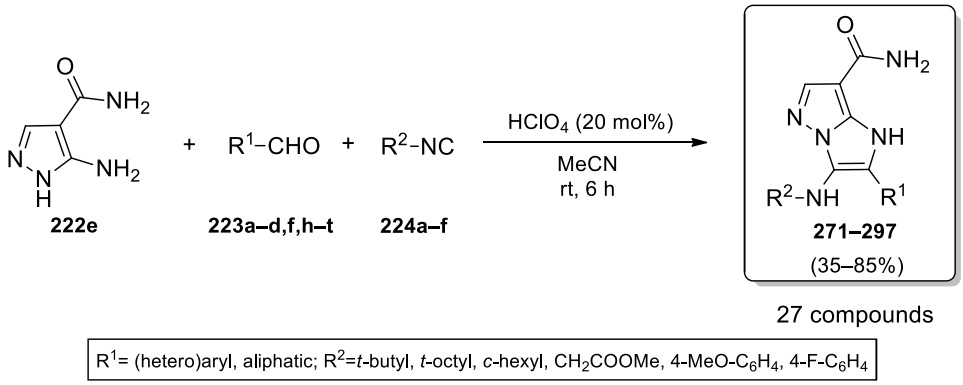
* Compound numbering is identical to that in the dissertation

3.3. The sequential one-pot two-step procedure was extended towards novel multisubstituted imidazo[1,2-*b*]pyrazole-7-carbonitriles and ethyl esters as well, starting from the appropriate **220b–d** compounds (Scheme 3). The *in situ* formation of **222b–d** aminopyrazoles required higher temperature (120 or 150 °C) to take place achieved by a 10-minute microwave irradiation. We observed that, while the electron-donating methyl substituent (R^1= CH_3) has a beneficial effect on the reaction, the replacement of the R^2 nitrile function to an ethyl ester had no significant influence on reaction yields.



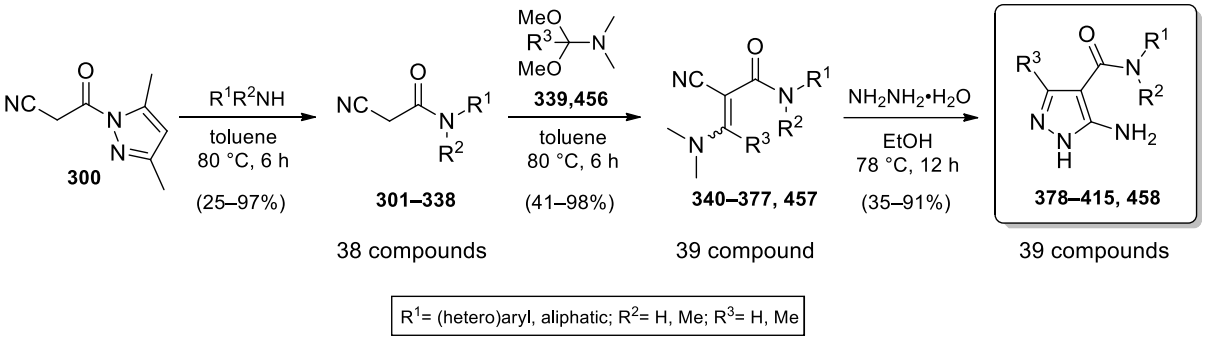
Scheme 3

3.4. The GBB reaction between 5-aminopyrazole-4-carboxamide (**222e**), aromatic or aliphatic aldehydes and isocyanides under modified reaction conditions (20 mol% $HClO_4$, MeCN, rt, 6 h) resulted in 27 novel imidazo[1,2-*b*]pyrazole-7-carboxamide derivatives (compounds **271–297**, Scheme 4). The utilization of aromatic aldehydes gave higher yields (46–85%) compared to their aliphatic counterparts (35–56%). The nature of the isocyanide component had no marked influence on the reaction.



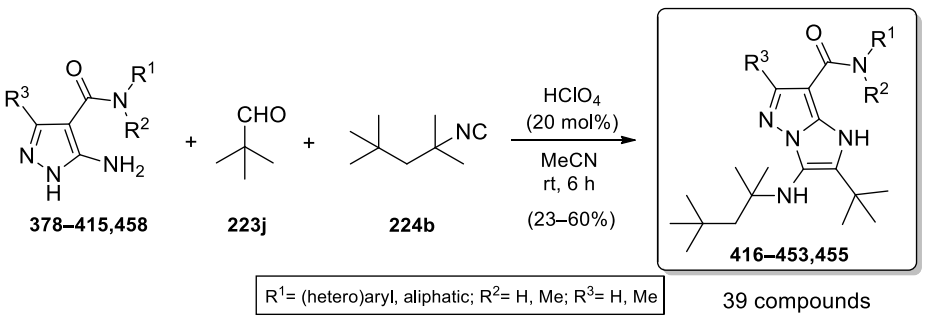
Scheme 4

3.5. For the extension of the imidazo[1,2-*b*]pyrazole-7-carboxamide library, 39 aminopyrazole derivatives (**378–415** and **458**) substituted at position C-4 by secondary and tertiary carboxamide moieties were prepared. In the three-step synthesis, first cyanoacetic acid derivative **300** was reacted with various amines in a nucleophilic substitution reaction to furnish cyanoacetamide analogues **301–338**. These were further transformed into the corresponding enamine derivatives (**340–377** and **457**) either by *N*,*N*-dimethylformamide dimethyl acetal (**339**) or *N*,*N*-dimethylacetamide dimethyl acetal (**456**). Finally, the ring-closing reaction of enamines and hydrazine monohydrate provided aminopyrazole-4-carboxamide products **378–415** and **458** (Scheme 5).



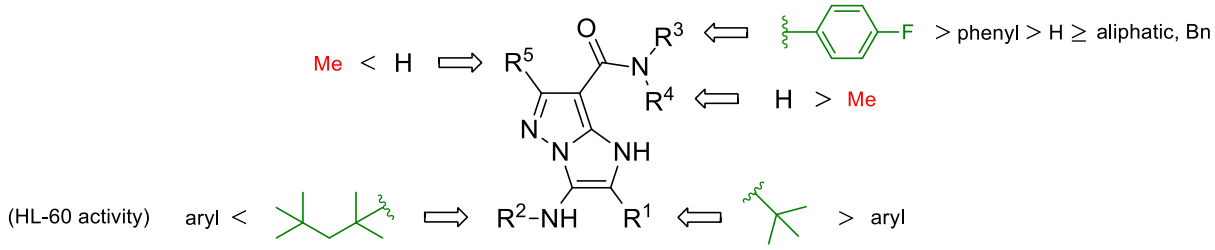
Scheme 5

3.6. In addition to imidazo[1,2-*b*]pyrazole products **271–297** described in section 3.4., the GBB reaction of aminopyrazole-4-carboxamides **378–415** and **458**, pivalaldehyde (**223j**) and *tert*-octyl isocyanide (**224b**) provided an additional group of 39 novel imidazo[1,2-*b*]pyrazole-7-carboxamide derivatives (Scheme 6). Moreover, an *N*-methyl-*N*-*tert*-octylamino analogue (compound **454**) was also synthesized via an Eschweiler-Clarke reaction. Products in this group were gained in moderate yields (23–60%).



Scheme 6

3.7. The synthesized imidazo[1,2-*b*]pyrazoles (**225–297** and **416–455**) were submitted to *in vitro* cytotoxicity tests at Avidin Ltd. on different human and mouse tumorous cell lines. In the case of certain imidazo[1,2-*b*]pyrazole-7-carboxamide derivatives, potent antitumor activity was found. On the basis of data from biological assays of imidazo[1,2-*b*]pyrazole-7-carboxamides (**271–297** and **416–455**), a detailed structure–activity relationship was established (Scheme 7). Among the primary carboxamide derivatives (**271–297**: R^3, R^4, R^5 = H), compound **292** (R^1 = *tert*-butyl, R^2 = *tert*-octyl) showed the highest antitumor activity. Compounds substituted with aromatic rings (R^1 and/or R^2 = aryl) exhibited potencies lower by one order of magnitude or were proved to be inactive. *N*-Alkyl or *N*-benzyl substitution on the carboxamide functionality of compound **292** resulted in diminished or similar activity (compounds **416–423**), while the introduction of a phenyl moiety had a positive effect on cytotoxicity against HL-60 cell line. This positive effect was further increased with a *p*-fluorophenyl substituent shifting the potency of compound **440** (R^1 = *tert*-butyl, R^2 = *tert*-octyl, R^3 = 4-F-C_6H_4, R^4, R^5 = H) into the nanomolar range on HL-60 cell line. Modifications on lead molecule **440**, like *N*-methylation on the *tert*-octylamino (R^2NH) moiety (compound **454**), establishing a tertiary carboxamide functionality (**453**: R^1 = *tert*-butyl, R^2 = *tert*-octyl, R^3 = 4-F-C_6H_4, R^4 = Me, R^5 = H) or the presence of a 6-methyl group (**455**: R^1 = *tert*-butyl, R^2 = *tert*-octyl, R^3 = 4-F-C_6H_4, R^4 = H, R^5 = Me) resulted in the drop or complete loss of cytotoxic activity.

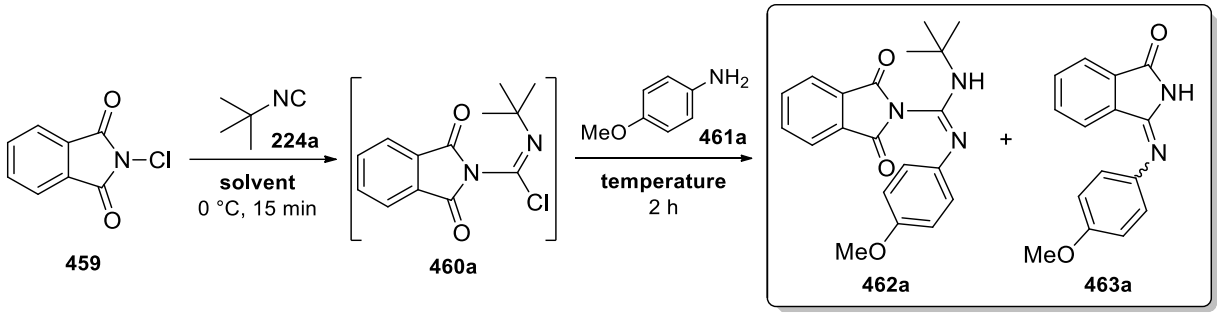


Scheme 7

3.8. In the third part of my experimental work, a sequential one-pot isocyanide-based method was developed for the synthesis of *N,N'*-disubstituted guanidines.

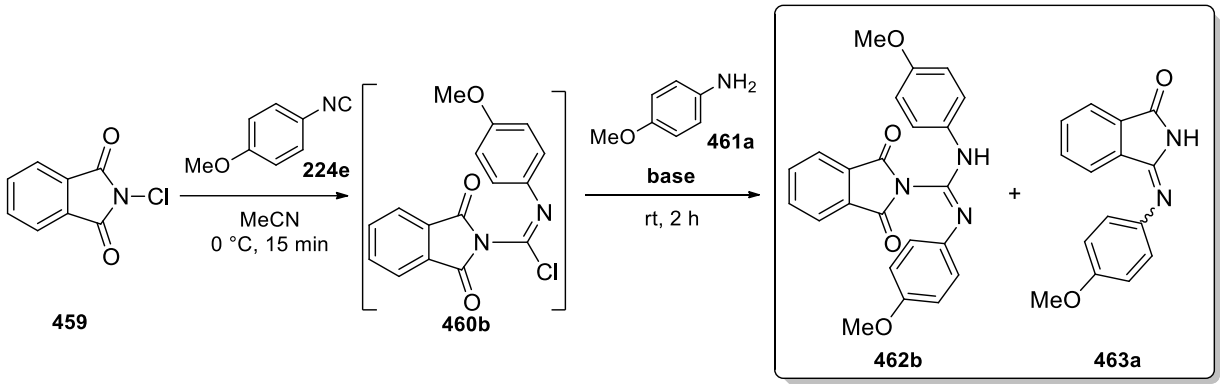
The feasibility to synthesize *N*-phthaloylguanidines was investigated in a model reaction employing *N*-chlorophthalimide, isocyanides and amines in a sequential one-pot two-step process. In the reaction using *tert*-butyl isocyanide (**224a**) and *p*-anisidine (**461a**), isoindolinone **463a** was also formed besides the expected *N*-phthaloylguanidine **462a** (Scheme 8). Optimization of reaction conditions revealed a marked solvent effect: apolar and ether-type

solvents delivered mainly isoindolinone **463a**, whereas polar aprotic media favored the formation of guanidine **462a** as the main product. The solvent of choice was found to be dry acetonitrile (**462a** in 75% HPLC yield). The substitution step was found to be best performed at room temperature. Neither lowering nor raising the temperature increased the yield.



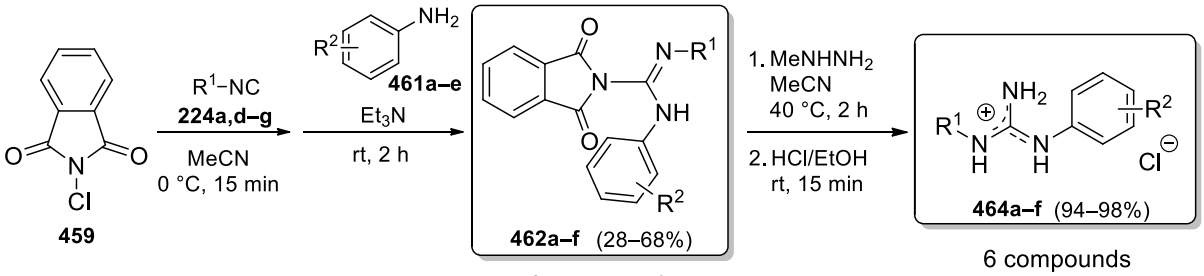
Scheme 8

3.9. The extension of the reaction for aromatic isocyanides was also investigated. Performing the model reaction under optimized conditions (MeCN solvent, room temperature after addition of **461a**) with 4-methoxyphenyl isocyanide (**224e**), the formation of isoindolinone **463a** was observed instead of the expected *N*-phthaloylguanidine **462b** (Scheme 9). While analyzing the individual reaction steps, we found that the reaction between *N*-chlorophthalimide and isocyanide **224e** gave stable and isolable adduct **460b**. We noticed that the addition of a suitable base (and *p*-anisidine **461a**) to the reaction mixture after the *in situ* formation of imidoyl chloride **460b** did facilitate the substitution step by neutralizing the liberated HCl. The reagent of choice proved to be triethylamine (TEA) (**462b** in 48% HPLC yield). A correlation between the basicity of the applied base and product ratio (**462b**:**463a**) was not found.



Scheme 9

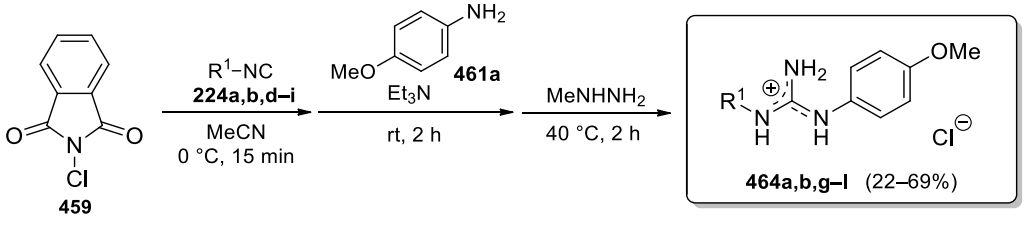
3.10. Following the optimized protocol (MeCN, 0 °C to rt, TEA in the second step) and employing aliphatic, benzyl and aromatic isocyanides (**224a,d–g**) together with electron-poor and electron-rich anilines (**461a–e**), six *N*-phthaloylguanidine derivatives (**462a–f**) with diverse electronic properties were synthesized (28–68%). Subsequently, the cleavability of the phthaloyl group was investigated by reacting **462a–f** with methylhydrazine at 40 °C for 2 hours while full conversion to *N,N'*-disubstituted guanidines **464a–f** was achieved (Scheme 10). The substitution pattern of compounds **462a–f** had no influence on the transformation and guanidines **464a–f** were isolated in excellent yields (94–98%, as HCl salts for the ease of isolation).



462a,464a: R^1=*t*-Bu, R^2=4-MeO-C_6H_4; **462b,464b**: R^1=4-MeO-C_6H_4, R^2=4-MeO-C_6H_4; **462c,464c**: R^1=4-MeO-C_6H_4, R^2=4-Br-C_6H_4; **462d,464d**: R^1=*c*-hexyl, R^2=3,5-di-Me-C_6H_4; **462e,464e**: R^1=Bn, R^2=4-F-C_6H_4; **462f,464f**: R^1=4-F-C_6H_4, R^2=C_6H_5

Scheme 10

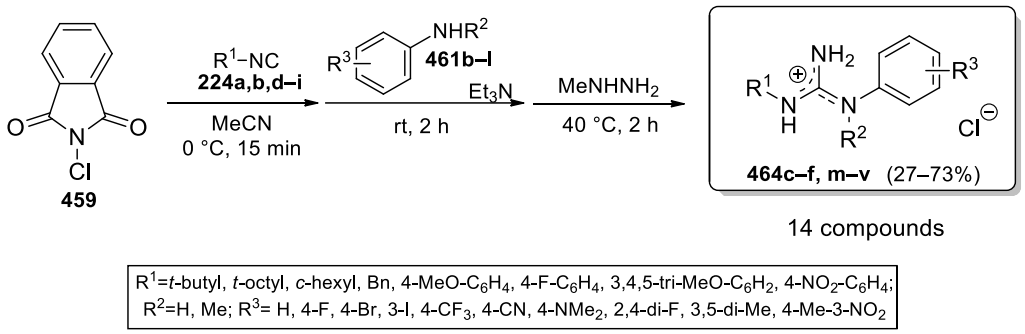
3.11. The synthesis of *N,N'*-disubstituted guanidines was further developed to a sequential three-step one-pot protocol omitting the isolation of *N*-phthaloylguanidines. First, eight *N,N'*-disubstituted guanidines were prepared by combining aliphatic and aromatic isocyanides (**224a,b,d–i**) and *p*-anisidine (**461a**) (Scheme 11). We noticed that the nucleophilic character of isocyanides had a significant effect on product yields. The best isolated yields were achieved with benzyl and aliphatic isocyanides (51–69%), while their aromatic counterparts gave inferior yields (22–48%). Additionally, in the case of aromatic isocyanides, the formation of *N*-aryl-*N'*-(4-methoxyphenyl)carbamides was also experienced besides isoindolinone **463a**.



R^1=*t*-butyl, *t*-octyl, *c*-hexyl, Bn, 4-MeO-C_6H_4, 4-F-C_6H_4, 3,4,5-tri-MeO-C_6H_2, 4-NO_2-C_6H_4

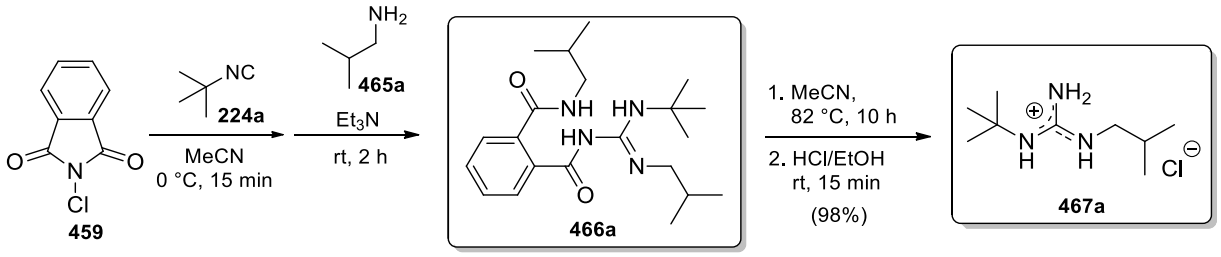
Scheme 11

3.12. Application of the sequential one-pot three-step method for both electron-rich and electron-poor anilines (**461b–l**) combined with isocyanides (**224a,b,d–i**) furnished 14 *N,N'*-disubstituted guanidines (27–73%) (Scheme 12). The electronic property of the aniline substituent (R^3), apart from the nitro group, had no marked influence on the reaction. The synthesis of compound **464n** (R^2 = Me) demonstrated that the method is suitable to prepare *N,N,N'*-trisubstituted guanidines as well. In the case of heteroaromatic amines (2-aminopyridine, 2-aminothiazole and 3-aminoisoxazole), the formation of the corresponding isoindolinone **463** side-products was observed instead of the desired guanidines.



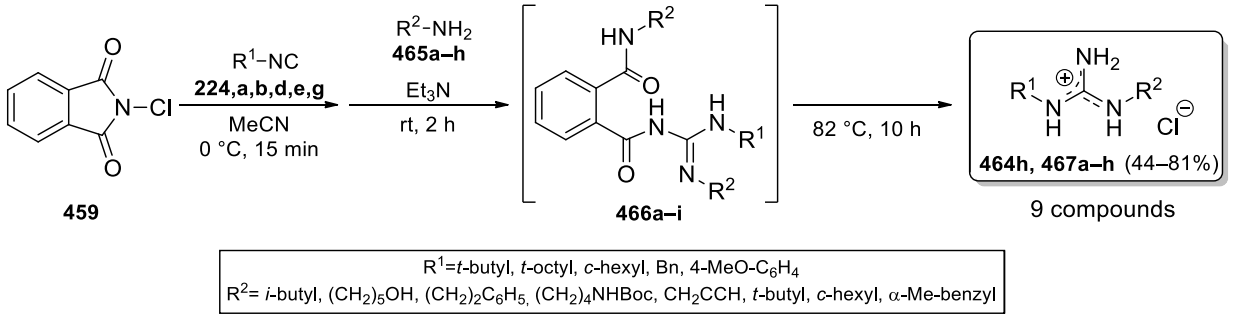
Scheme 12

3.13. We also examined the possible extension of the reaction for aliphatic amines. Surprisingly, the reaction of *N*-chlorophthalimide, *tert*-butyl isocyanide and isobutylamine gave product **466a**, which was interpreted by the *in situ* ring opening of the expected *N*-phthaloyl-guanidine by isobutylamine (Scheme 13). Since ring-opening could not be prevented even at low temperature (-40 °C), the synthesis of *N,N'*-disubstituted guanidine **467a** was achieved by an intramolecular nucleophilic substitution-type debenzoylation of **466a** omitting hydrazine from the sequence (Scheme 13).



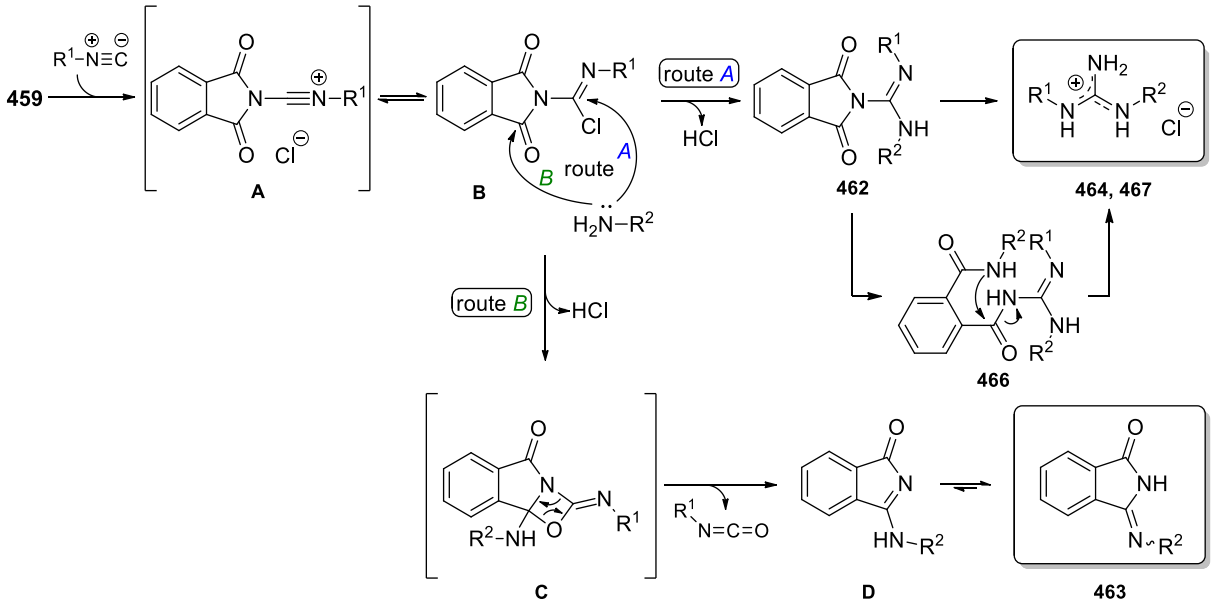
Scheme 13

3.14. In order to successfully utilize aliphatic amines, the sequential one-pot three-step protocol was modified. In the second step, 2.2 equivalents of amines **465** were used and intermediates **466** were simply transformed into *N,N'*-disubstituted guanidines **467** by heating. By applying this modified method, 9 guanidine derivatives were prepared (44–81%) from primary aliphatic and benzylamines (Scheme 14).



Scheme 14

3.15. A reaction mechanism was also proposed. The α-addition of isocyanide on *N*-chlorophthalimide furnishes imidoyl chloride intermediate **B** (Scheme 15). Amine attack may occur at either the imidoyl carbon (route *A*) or the phthalamide carbon (route *B*) resulting in the appropriate *N*-phthaloylguanidine **462** or isoindolinone **463**, respectively. On route *B*, a retro [2+2]-cycloaddition-type rearrangement furnishes isocyanate as a side-product (step **C** to **D**) confirmed in a control experiment. Intermediate **B** was also isolated in a control experiment providing further support to the mechanism.



Scheme 15

4. Scientific publications forming the basis of the doctoral dissertation

1. **A. Demjén**, M. Gyuris, J. Wölfling, L. G. Puskás, I. Kanizsai
   Facile synthesis of 1*H*-imidazo[1,2-*b*]pyrazoles *via* a sequential one-pot synthetic approach
   Beilstein J. Org. Chem. **2014**, *10*, 2338–2344.

   IF: 2.762

2. **A. Demjén**, A. Angyal, J. Wölfling, L. G. Puskás, I. Kanizsai
   One-pot synthesis of diverse *N,N′*-disubstituted guanidines from *N*-chlorophthalimide, isocyanides and amines via *N*-phthaloyl-guanidines
   Org. Biomol. Chem. **2018**, *16*, 2143–2149.

   IF: 3.564
   (2017)

3. **A. Demjén**, R. Alföldi, A. Angyal, M. Gyuris, L. Hackler, G. Szebeni, J. Wölfling, L. Puskás, I. Kanizsai
   Synthesis, Cytotoxic Characterization, and SAR Study of Imidazo[1,2-*b*]pyrazole-7-carboxamides
   Arch. Pharm. Chem. Life Sci. **2018** *(accepted for publication, DOI: 10.1002/ardp.201800062).*

   IF: 1.994
   (2017)

Total IF: 8.320

5. Scientific lectures and posters forming the basis of the doctoral dissertation

1. **A. Demjén**, M. Gyuris, L. G. Puskás, J. Wölfling, I. Kanizsai
   Synthesis of Imidazo[1,2-*b*]pyrazole Derivatives via GBU Reaction (poster)
   9th International Congress of Young Chemists, Kraków (Poland), **2011**.

2. Gyuris M., **Demjén A**., Madácsi R., Puskás G. L., Wölfling J., Kanizsai I.
   Imidazo[1,2-*b*]pirazol származékok előállítása GBU reakcióval (poster)
   MKE 1. Nemzeti Konferencia, Sopron (Hungary), **2011**.

3. **Demjén A.**
   Tumorellenes hatású imidazo[1,2-*b*]pirazol molekulakönyvtár Groebke-Blackburn-Bienaymé reakción alapuló négykomponensű szintézise (lecture)
   Heterociklusos és Elemorganikus Kémiai Munkabizottság ülése, Balatonszemes (Hungary), **2012**.

4. **A. Demjén**, L. G. Puskás, J. Wölfling, I. Kanizsai
   A facile three-component assembly of trisubstituted *N*,*N*-phthalylguanidine species (poster)
   14th Belgian Organic Synthesis Symposium, Louvain-la-Neuve (Belgium), **2014**.

5. **Demjén A.**
   Az *N*-klórftálimidtől a guandinek felé (lecture)
   Szegedi Ifjú Szerves Kémikusok Támogatásáért Alapítvány 15. tudományos előadóülése, Szeged (Hungary), **2016**.

6. **A. Demjén**, A. Angyal, L. G. Puskás, J. Wölfling, I. Kanizsai
   Novel Isocyanide-Based Approach for the Synthesis of *N,N'*-Disubstituted Guanidines through *N*-Phthaloylguanidines (poster)
   27th European Colloquium on Heterocyclic Chemistry, Amsterdam (Netherlands), **2016**.

7. G. J. Szebeni, **A. Demjén**, I. Kanizsai, L. Hackler Jr., L. G. Puskás
   Small molecules DU192, DU283 and DU325 induce differentiation and apoptosis of human acute promyelocytic leukemia cells (poster)
   Magyar Immunológiai Társaság 46. Vándorgyűlése, Velence (Hungary), **2017**.

8. G. J. Szebeni, A. Demjén, I. Kanizsai, L. Hackler Jr., L. G. Puskás
   Imidazo[1,2-*b*]pyrazole-7-carboxamides: DU192, DU283 and DU325 induce differentiation and apoptosis of human acute promyelocytic leukemia cells (poster)
   CYTO, Prague (Czech Republic), **2018**.

6. Scientific publications not related to the doctoral dissertation

1. P. Bata, **A. Demjen**, F. Notheisz, A. Zsigmond
   Comparative study of immobilized phthalocyanines in oxidative degradation
   Open Catal. J. **2012**, *5*, 50–55.

   IF: –

2. A. Angyal, **A. Demjén**, J. Wölfling, L. G. Puskás, I. Kanizsai
   A green, isocyanide-based three-component reaction approach for the synthesis of multisubstituted ureas and thioureas
   Tetrahedron Lett. **2018**, *59*, 54–57.

   IF: 2.193
   (2017)

3. A. Angyal, **A. Demjén**, E. Wéber, A. K. Kovács, J. Wölfling, L. G. Puskás, I. Kanizsai
   Lewis acid-catalyzed diastereoselective synthesis of multisubstituted *N*-acylaziridine-2-carboxamides from 2*H*-azirines *via* Joullié-Ugi three-component reaction
   J. Org. Chem. **2018**, *83*, 3570–3581.

   IF: 4.849
   (2017)

Total IF: 7.042